Development of an image algorithm to assess changes in leaf pigments content of fresh-cut spinach leaves

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Abstract

The aim of this work was to develop an image algorithm to detect changes in colour related with changes in leaf pigments content in leafy spinach during storage. The experiment was carried out on packed ready-to-use spinach stored at 4.5 °C. Seventy-five leaves of spinach were analyzed at zero time and after storage for 7, 14 and 21 days. Multispectral images were acquired in the red (R), infrared (IR) and blue (B) regions. Virtual images were calculated on the basis of spectral indexes usually employed for estimation of leaf pigment content. By considering the sensitive bands to chlorophyll and carotenoids, new virtual images were proposed. A no supervised classification was applied to the obtained virtual images and the results were evaluated according to colorimetric measurements (CIE *L*a*b** coordinates). The new proposed indexes were able to correctly classify a high percentage of the samples according to the colour evolution.

Key words: *Ready-to-use; green leaves; machine vision.*

1. Introduction

In recent years, consumption of fresh vegetables has been increasing, especially as a result of changes in the life style of the consumers. In particular, ready-to-use (RTU) or minimally processed vegetables industry has growth a lot because of the increasing of the demand for fresh, healthy and convenient foods (Ragaert et al. 2004). RTU vegetables typically involve peeling, slicing, dicing or shredding prior to packaging and storage (Beuchat 1996). It is well-known that processing of vegetables promotes a faster physiological deterioration (membrane deterioration, tissue softening, water loss, susceptibility to microbiological spoilage), biochemical changes (such as chlorophyll, carotenoids and polyphenols degradation, ethylene production, oxygen consumption and carbon dioxide production, loss of acidity, increase in sweetness, formation of flavour volatiles, lipolysis and lipid oxidation) (Toivonen et al. 2002) and microbial degradation of the product (O'Beirne et al. 2003). All these phenomena determine a rapid quality decay of RTU products compared to whole products, which may results in degradation of the colour, texture, flavour and nutritional value (Kabir 1994; Varoquaux et al. 1994). For this reason, most of these products are sold within 1 week after packaging (Rolle et al. 1987).

The postharvest techniques for preserving the quality of fresh-cut vegetables are aimed at maintaining external colour and internal phytonutrient content, preventing tissue browning and improving hygiene (Brecht 1995). Disorders arising from processing can be minimized by the use of sharp cutting tools, enzymatic browning inhibitors, modified atmospheres and low temperatures (Watada et al. 1999; Jacxsens et al. 2002). It is well known that low temperatures slow down plant metabolic processes such as respiration, ethylene production and, in general, enzyme activity. However, low temperatures may induce chilling injury and compromise product quality. Correct storage temperature can vary from species to species and cultivar to cultivar. The most frequently used temperature is 4 °C, considered the optimal for many leafy vegetables (Jacxsens et al. 2002). In spite of this, the published data suggest that none of the available washing and sanitizing methods can guarantee the microbiological quality of minimally processed vegetables without compromising their sensorial quality (Beuchat et al. 1998).

Plant pigments determination

Quality of RTU fruit and vegetable determines the value to the consumer and it is a combination of parameters including appearance, texture, flavour and nutritional value (Kader 2002). Since consumers judge quality of fresh-cut products on the basis of appearance and freshness at time of purchase, the colour is extremely important (Ferrante et al. 2004). RTU vegetables can undergo changes in colour due to different biochemical processes involving plant pigments that have a great importance for leaf function. For this reason, variations in pigment content (e.g. carotene and chlorophyll degradation, oxidation of phenolic compounds, etc.) may provide information concerning the physiological state of leaves. Plant pigments content can be evaluated through traditional methods (e.g. chemical extraction), which require destruction of the samples, are time-consuming and labour intensive, or through non destructive techniques, such as spectrophotometric determinations, which provide a fast and non-destructive method for pigment estimation. A large number of spectral indices have been developed for estimation of leaf pigment content. Regarding to the visible (VIS) spectral range, the major part of these indices employ wavelengths belonging to the red optical range (R), between 660 and 700 nm, because there is one of the absorbance peak of chlorophyll, the range 450-480 nm and near 550 nm, because carotenoids and anthocyanins absorb particularly strongly in the blue and green regions (Sims et al. 2002; Qin et al. 2008). Concerning to the infrared region of the spectrum, the near-infrared (NIR) is the most useful area to estimate chlorophyll content (Xue et al. 2008). By combining IR and R, two major indexes have been developed: the well known *Normalized Difference Vegetation Index* (NDVI), that is IR-R/IR+R, and the R/IR Index (Bodria et al. 2004; Lleó et al. 2009). Since spectroscopic techniques can analyze only small portions of the product (coinciding with the size of the optical fibre) and require the repetition of the analysis in different areas of the product, it could be interesting to estimate the leaf pigment content through a multispectral vision system based on the cited sensitive bands.

Aims and objectives of the research

The objective of the present research was to calculate virtual images based on the most sensitive spectral regions to caretoinds and chlorophyll, which were able to emphasize the spectral changes related to plant pigments content. The aim of the work was to develop an image algorithm to classify RTU leafy spinach according to changes in colour during storage and the final purpose was to identify the best virtual image to discriminate between freshness stages along refrigerated storage.

2. Materials and methods

Plant material

The experiment was carried out on RTU leafy spinach (*Spinacia oleracea*), harvested from an orchard in Murcia (Spain) and packed in sealed plastic bags filled with 500 g. The packed spinaches were stored at 4.5 °C and 85 % HR for 21 days. A leaf of spinach was considered as sample units in this work. Seventy-five units were evaluated per day: at zero time (treatment t_0) and after 7 (treatment t_1), 14 (treatment t_2) and 21 (treatment t_3) days of storage.

Vision System

Images were acquired through a IRRB camera (DuncanTech/Redlake MS-3100®, Redlake Inc., USA) with a digital resolution of 1300×1000 pixels, endowed with three band-pass filters (band-width: 20 nm), centred at 800 nm (IR), 680 nm (R) and 450 nm (B) and three monochromatic images can be analyzed from each sample: IR, R and B. The light source was provided by six 100W/220V halogen lamps. The images were acquired using a black background and a black canvas, in order to create a uniform light field around the object and to eliminate any effect of environmental light.

Reflectance spectra and colour parameters

By employing a portable spectrophotometer Minolta CM-50I (Konica Minolta Sensing, Inc., Japan), visible relative reflectance spectra 360-740 nm, at 10 nm intervals, were obtained and CIE $L^*a^*b^*$ coordinates were measured for each sample, where L^* is the luminance component and a^* and b^* are colour coordinates related respectively with the red/green and yellow/blue spectral ranges (Yam et al. 2004). For each sample the mean of triplicate measurements, performed on one side of each leaf, was evaluated. A standard white calibration plate was employed to calibrate the equipment.

Spectral considerations about VIS spectra of spinach leaves

Fig. 1 presents a reflectance spectrum of a leaf of spinach stored ad 7.5 ºC at the beginning (t_0) and at the end (t_1) of the shelf-life. The main differences are that the relative reflectance values of t_0 spectrum are always higher than those of t_1 along the all VIS wavelengths and that the spectrum acquired at the beginning of the storage period presents a peak in the green/yellow areas (500-580 nm approximately), that decreases at the end of the storage period. Since the camera employed in this work was not equipped with filters working in these ranges but was endowed with their complementary colours, respectively red (660-700 nm) and blue (430-470 nm), virtual images based on these wavelengths were employed in order to test their feasibility to emphasize the observed changes in the spectral features in the green/yellow areas. Besides, how the above considerations suggest, the red and the blue regions are closely related to chlorophyll (red region) and to caretonoids content (blue range).

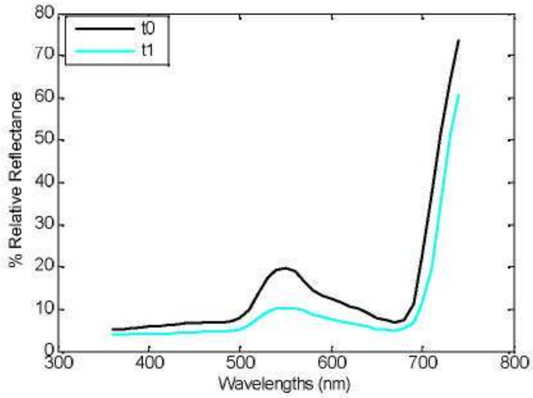

Fig. 1: VIS relative reflectance spectra belonging to a leaf of spinach at the beginning and at the end of the shelf-life.

Image processing

An IRRB image was acquired for each sample and processed off–line in Matlab® (MathWorks, Inc., USA). Spinach samples were distinguished from the background by performing a segmentation according to *Otsu method* (Otsu 1979) on the R images. The resulting binary image was multiplied by the images acquired at 800 (IR), 680 (R) and 450 (B) nm and by the following virtual images: R/IR (R divided by IR), IR-R/IR+R (corresponding to the *Normalized Difference Vegetation Index*, NDVI), B/R (B divided by R) and R-B/R+B. How above explicated, the last two image combinations were chosen in order to emphasize the possible changes in the spectral features related to the red (660-700 nm) and to the blue regions (450-480 nm). Further analysis was based on the relative histograms of the above virtual images. In what follow, Ind_1 refers to R/IR virtual image, Ind_2 to NDVI, Ind_3 to B/R and Ind_4 to R-B/R+B.

Cluster analysis

A hierarchical cluster analysis according to Ward's method was performed in order to define *reference classes* (RC) based on the histograms of the digital images acquired from the samples. The following procedure was applied for each one of the indexes, *i.e.* Ind_1, Ind_2, Ind_3 and Ind_4: relative histograms of all the samples were calculated; all the histograms intensity levels were considered as a dimension of a multidimensional space, where a single histogram was represented as a single point; the matrix of Euclidean distances between each pair of individuals (histograms) was computed in order to group the closest ones and to hierarchically merge individuals whose combination gave the least *Ward Linkage distance* (that is the minimum increase within sum of squares of the new-formed group); the groups were generated on the basis of an input maximum *Ward linkage distance*; the average histogram was computed for each generated group and defined as RC.

Internal validation

An internal validation was carried out by classifying again the same population generating the model into the previously generated RC (each one defined by the average histogram of the class) to which it computed the minimum *Euclidean distance* (E_d). The *observed* classification was compared with the *predicted* classification to the RC based on the minimum E_d.

Statistical analysis

The CIE *L*a*b** coordinates were employed as a reference of changes in colours during storage with regard to multispectral image information. ANOVA analysis was applied to the colour parameters and to the classes extracted from the image analysis and the results were used to evaluate the classification based on the histograms of virtual images of each index.

3. Results

Generation of browning reference classes

First column of Fig. 2 shows, for each virtual image, the average histograms calculated for each treatment. It is possible to note that the average histograms corresponding to the first and the second treatment (t_0 and t_1) were quite close between them and appeared separated from the average histograms of the images acquired on the 14th and 21st day. This observation was confirmed by the cluster analysis results, graphically reported (as dendrogram) in the second column of the Fig. 2: by setting the maximum *Ward Linkage distance* within groups at 0.45 pixel relative frequency, in the case of Ind_1, at 0.55 for Ind_2 and at 0.40 for Ind_3 and Ind_4, two clusters, corresponding to two RC (Class A and Class B), were obtained for each virtual image.

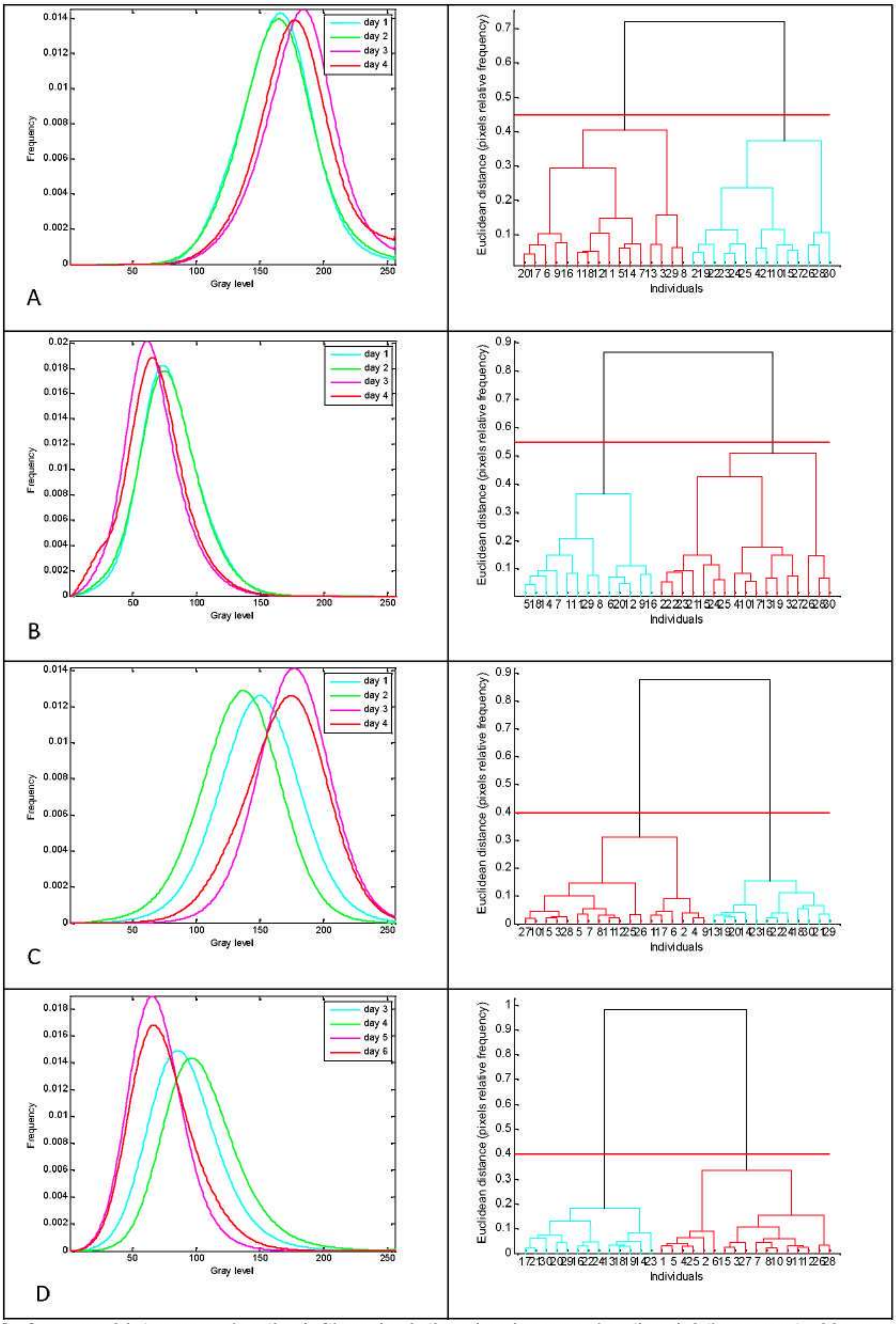

Fig. 2: Average histograms (on the left) and relative dendrogram (on the right) generated by applying Ward's non supervised classification to all digital images of the samples based on Ind_1 (panel A), Ind_2 (panel B), Ind_3 (panel C) and Ind_4 (panel D). Horizontal lines in the cluster trees represent the maximum Ward Linkage distance within groups.

Table 1 reports the classification matrix obtained by applying the cluster analysis to each image combination. The non-supervised classifications based on the Ind_3 and Ind_4virtual images were able to segregate quite well between the storage periods, since in both of cases near 93 % of Class A was composed by samples measured at zero time and after 7 days of storage, and near 98 % of Class B was composed by samples analyzed on the 14th and 21th days. On the otherwise, the classifications based on the Ind_1 and Ind_2 did no segregate so well the storage periods; Class A included respectively 67 and 77 % of samples analyzed on the first two days and Class B comprised 88 and 76 % of samples analyzed on the last two ones.

Tab. 1: Classification matrix of spinach samples: treatments (observed classification) against reference camera classification (predicted classification).

		OBSERVED	
	Classes \ Treatments	t_0- t_1	t_2-t_3
		Ind $_1$ = R/IR	
	A	128	62
	B	22	88
		Ind $_2$ = NDVI	
PREDICTED	**A**	114	33
	B	36	117
		Ind $_3$ = B/R	
	A	146	12
	B	4	138
		Ind $_4$ = R-B/R+B	
	A	148	13
	B	2	137

Internal validation

Regarding the internal validation of the model, in the case of Ind_3 and Ind_4 histograms, near 94 % and 97 % of the samples were classified in the same group by both methods: the Ward's non supervised classification and the classification according to Euclidean distances to Ward generated reference classes. For *Ind_1 and Ind_2* histograms, the two methods classified near 85 % of samples in the same group (Table not shown).

Statistic analysis of colour coordinates

Table 2 reports the average values and confidence intervals (95%) of CIE $L^*a^*b^*$ colour coordinates for each image based class. In all cases, an increase in storage time was accompanied by an increase in colorimetric a^* and b^* values and a decrease in lightness (L^*). These results are according to previous works that studied colour coordinates evolution during storage (Perez-Gago et al. 2006; Lu et al. 2007). Table 2 also reports the results of ANOVA Fisher Least Significant Differences (LSD) test performed on L^*, a^* and b^* colour coordinates of the samples classified in Classes A and B according to Ward's method based on Ind_1, Ind_2, Ind_3 and Ind_4. It is shown a consistent increase in a^* values and a consistent decrease in L^* values from Class A to B. Concerning to b^* parameter, class A and B are not significantly different and could be grouped in the same class. According to the F values, the classification based on Ind_3 and Ind_4 showed the best accordance with the colour parameters.

Tab. 2: Average values, confidence intervals (95%) and results of ANOVA Fisher LSD test performed on L*, a* and b* colour coordinates of samples grouped in classes A and B according to Ward's method based on Ind_1, Ind_2, Ind_3 and Ind_4. Three asterisks mean that there is significant difference (p<0.001) between means, while n.s. implies there is no significant difference.

			Ind_1			
Colour Coordinates	*L*mean*	$\pm SD_{L^*}$	*a*mean*	$\pm SD_{a^*}$	*b*mean*	$\pm SD_{b^*}$
Class A	44.41	0.22	-9.86	0.06	21.58	0.21
Class B	43.20	0.43	-9.28	0.08	21.79	0.22
F-values	14.18***		15.5***		$3.75^{n.s}$	
			Ind_2			
Colour Coordinates	*L*mean*	$\pm SD_{L^*}$	*a*mean*	$\pm SD_{a^*}$	*b*mean*	$\pm SD_{b^*}$
Class A	44.63	0.24	-10.46	0.06	20.02	0.23
Class B	43.57	0.26	-9.99	0.07	21.28	0.25
F-values	13.67***		14.14***		$3.7^{n.s}$	
			Ind_3			
Colour Coordinates	*L*mean*	$\pm SD_{L^*}$	*a*mean*	$\pm SD_{a^*}$	*b*mean*	$\pm SD_{b^*}$
Class A	45.65	0.18	-11.03	0.06	21.01	0.23
Class B	43.04	0.20	-9.72	0.07	21.77	0.22
F-values	16.60***		23.13***		$3.23^{n.s}$	
			Ind_4			
Colour Coordinates	*L*mean*	$\pm SD_{L^*}$	*a*mean*	$\pm SD_{a^*}$	*b*mean*	$\pm SD_{b^*}$
Class A	45.70	0.22	-10.40	0.05	21.08	0.21
Class B	43.00	0.23	-8.98	0.08	21.79	0.23
F-values	16.77***		22.33***		$3.21^{n.s}$	

4. Conclusion

In the present work a new method employing a multispectral vision system to classify leafy spinach on the basis of changes in colours related to changes in plant pigments content during storage was presented.

The method utilized relative histograms of virtual images based on multispectral indexes, e.*g*. Ind_1, Ind_2, Ind_3 and Ind_4, combinations of infrared (IR, 800 nm), red (R, 680 nm) and blue (B, 450 nm) images. In particular, Ind_1 and Ind_2 employed the IR and the R spectral ranges and were derived from bibliography, while Ind_3 and Ind_4 were new proposed and utilized the R and the B ranges.

On the basis of classification procedure results, all the indexes were able to detect changes in colour, classifying samples into two reference classes (Class A to Class B), including respectively the major part of the samples analyzed on zero time and on the 7th day of storage and samples analyzed on the 14th and the 21th day. The indexes based on the red and the blue spectral ranges gave better results in the internal validation procedure than those based on infrared and red spectral range.

In all cases, Class A to B presented decreasing lightness and increasing *a** and *b** values, but image combination based on Ind_3 *and* Ind_4 showed the best sensitivity to reflect the change in colours associated with discoloration.

All these results confirm the red and blue spectral ranges (complementary to the green and the yellow colours, were the changes in the spectral features are more evident) contain enough information for segregating extreme stages. It could be used as a potential criterion for establishing the optimal shelf-life of RTU packed spinach under refrigeration condition. Further works should be carried out in order to improve the performance of the classification models, adding others wavelengths to generate the virtual images.

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